

Randomised Controlled Trial of meropenem versus piperacillin-tazobactam for definitive treatment of bloodstream infections due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* spp. (MERINO Trial)

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

Original Trial Protocol

STUDY PROTOCOL

PROTOCOL TITLE:

Randomised Controlled Trial of meropenem versus piperacillin-tazobactam for definitive treatment of bloodstream infections due to third generation cephalosporin non-susceptible *Escherichia coli* and *Klebsiella* spp. (MERINO Trial)

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STUDY PROTOCOL

1. BACKGROUND AND RATIONALE

1.1. General Introduction

Escherichia coli and *Klebsiella* spp. are common causes of bacteraemia, and may acquire genes encoding AmpC or extended-spectrum beta-lactamases (ESBLs). ESBL or AmpC producers are typically resistant to third generation cephalosporins such as ceftriaxone, but susceptible to carbapenems. In observational studies that have been performed to evaluate antibiotic choices for ESBL-producers, no agent has been shown to significantly surpass carbapenems. However, widespread use of carbapenems may cause selection pressure leading to carbapenem-resistant organisms, thus further limiting therapeutic options to “last-line” antibiotics such as colistin or tigecycline. Some new beta-lactam antibiotics and beta-lactamase inhibitors, which are active against ESBL, AmpC and some carbapenemase producing organisms, are in advanced clinical development. However, these antibiotics are likely to be expensive, have not yet made it to market and may best be held in reserve for infections where there are no alternatives. Therefore, we see a need for establishing the efficacy of a generically available alternative to carbapenems for serious infections.

The susceptibility of AmpC and ESBL producers to piperacillin/tazobactam is less predictable than that of carbapenems. By definition, ESBLs are inhibited by beta-lactamase inhibitors such as tazobactam. However, *E. coli* or *Klebsiella* may produce multiple beta-lactamase types some of which are resistant to inhibition by tazobactam. There have also been concerns that inoculum effects may overwhelm the activity of beta-lactamase inhibition in infections with a large bacterial burden. Additionally, in some cases outer membrane protein loss may contribute to resistance to tazobactam. By definition, AmpC is not inhibited by beta-lactamase inhibitors such as tazobactam. However, despite these limitations, approximately 50% or more of ceftriaxone non-susceptible *E. coli* or *Klebsiella* remain susceptible in vitro to piperacillin/tazobactam.

No randomised controlled trials have yet been performed comparing different treatment options for third generation cephalosporin-resistant *Enterobacteriaceae*. The largest observational study with an analysis by treatment outcome was published in February 2012 by Rodriguez-Bano *et al.* They performed a post-hoc analysis of six published cohorts of patients with bacteraemia due to ESBL producing *E. coli*. Two non-mutually exclusive cohorts (empirical therapy and definitive therapy) were constructed and analysed separately. In both cohorts, carbapenems were not superior to beta-lactam/beta-lactamase inhibitor (BLBLI) combinations. Specifically, in the definitive therapy cohort, mortality rates at 30 days were not significantly different – 9.3% for those who received a BLBLI and 16.7% for those who received a carbapenem ($p>0.20$).

Both meropenem and piperacillin-tazobactam are antibiotics that have been widely used in clinical practice for many years. They have proven efficacy in wide range of infectious syndromes, including severe sepsis, febrile neutropenia, ventilator-associated pneumonia and intra-abdominal sepsis. Meropenem is a carbapenem that is able to resist the action of a wide range of bacterial hydrolytic enzymes, including broad-spectrum types such as AmpC and

ESBLs. Piperacillin/tazobactam combines a broad-spectrum penicillin (piperacillin) with a beta-lactamase inhibitor (tazobactam). Both agents are licenced for the treatment of serious infections in Singapore and are available via the hospital pharmacy for routine clinical use.

1.2. Rationale and justification for the Study

Our hypothesis is that piperacillin/tazobactam is non-inferior to meropenem for the definitive treatment of bloodstream infections due to third-generation cephalosporin non-susceptible *E. coli* or *Klebsiella* spp.

The study design will be a randomised controlled multicentre trial. Both study drugs (meropenem and piperacillin/tazobactam) will administered intravenously with standard optimised dosing regimens. The study population will be all adult patients (21 years of age or older) admitted to any NUH ward. Inclusion in the study will be determined by the presence of a bloodstream infection with *E. coli* or *Klebsiella* spp., as defined by at least one positive blood culture from a peripheral blood draw, where the isolate is confirmed to be 3rd generation cephalosporin non-susceptible, but susceptible to piperacillin/tazobactam and meropenem

Meropenem 1 gram will be administered every 8 hours intravenously or piperacillin/tazobactam 4.5 grams administered every 6 hours intravenously. Each dose will be given over 30 minutes. The study drug is to be administered for a minimum of 4 days and can be given for as long as 14 days. The duration of therapy will be determined by the treating clinician.

a. Rationale for the Study Purpose

No randomised controlled trials (RCTs) have yet been performed comparing different treatment options for AmpC or ESBL-producing *Enterobacteriaceae*. During the last 10 years we have seen an exponentially increasing rate of carbapenem resistance worldwide, including Singapore. We urgently need data from well-designed RCTs to guide clinicians in the treatment of antibiotic resistant Gram-negative infections. We face a situation where a commonly used antibiotic for these infections (meropenem) may be driving carbapenem resistance. For this reason, we are seeking to compare a carbapenem-sparing regimen with a carbapenem for the treatment of these infections. Formal evaluation of safety and efficacy of generic antibiotics in the treatment of infection is of immense clinical and public health importance, and no formal trial has yet been conducted to address these issues. The international collaboration between teams of clinician researchers, some of whom are leaders in their field, make it highly likely that the outcomes of this trial will have a significant impact on clinical practice.

b. Rationale for Doses Selected

The dosing regimens selected are the standard schedules for serious infections for both agents. For piperacillin/tazobactam, the use of 4.5g given every 6h (as opposed to 8 hourly) has been shown to have a more favourable pharmacokinetic/pharmacodynamic (PK/PD) profile for treating Gram-negative organisms where the MIC is at the higher end of the susceptible range or just below the breakpoint.

c. Rationale for Study Population

The aim of the study is to help clinicians and microbiologists make evidence-based therapeutic decisions for patients with serious infections caused by ESBL producers. As ESBL infections

can be seen in a large range of clinical situations, spanning all departments and patient populations, the study logically requires recruitment of patients from all areas of the hospital system. In general, these infections are usually treated in admitted patients (due to their severity), and so inclusion of out-patients is not applicable.

d. Rationale for Study Design

The optimal study design to answer whether one drug (piperacillin/tazobactam) is non-inferior in comparison to another (meropenem) is a randomised controlled trial (RCT). To date, no RCTs have yet been performed in this specific area. The common recommendation for the use of carbapenems is based on non-randomised observational studies, which always have the inherent problems of bias and confounding factors. Such methodological issues always weaken interpretations derived from these studies. A well-conducted RCT will provide the highest possible level of evidence upon which to base clinical decision-making.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

Our hypothesis is that piperacillin/tazobactam (a carbapenem-sparing regimen) is non-inferior to meropenem (a widely used carbapenem) for the definitive treatment of bloodstream infections due to third-generation cephalosporin non-susceptible *E. coli* or *Klebsiella* spp.

2.2. Primary Objectives

To compare the 30-day mortality post bloodstream infection of piperacillin/tazobactam and meropenem.

2.3. Secondary Objectives

- (1) To compare the time to clinical and microbiologic resolution of infection for each regimen;
- (2) To compare the clinical and microbiologic success of each regimen at day 4 of the intervention;
- (3) To compare the risk of relapse with each regimen;
- (4) To compare the risk of superinfection with a carbapenem resistant organism with each regimen

2.4. Potential Risks and benefits:

a. End Points - Efficacy

Retrospective studies suggest that piperacillin/tazobactam is non-inferior to meropenem in the treatment of ESBL-producing bacteria. Therefore, we do not anticipate that either drug will provide specific benefits or additional efficacy. However, piperacillin/tazobactam may provide less selective pressure for colonisation and infection with carbapenem-resistant bacteria.

b. End Points - Safety

The main risk to the study population is that piperacillin/tazobactam may prove inferior to meropenem in the treatment of ESBL-producers. However, both drugs are currently commonly used in clinical practice for this indication when *in vitro* susceptibility is reported. We therefore, do not believe that we are exposing patients to excess risk by study inclusion beyond the risks involved in standard therapeutic decisions and clinical management.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

Although the study is a multi-centred study, involving 8 institutions across Australia, New Zealand and Singapore, NUH is the only study site in Singapore. The study population will be drawn from any patient admitted to NUH with a blood culture positive for third generation cephalosporin non-susceptible *E. coli* or *Klebsiella*. There will be no restrictions on race, gender or ethnicity. Minors (aged <21y) have been excluded both to simplify the consent procedure and since the response to infection in children may be different than in adults (for instance, shorter duration of therapy is commonly used). We aim to recruit 310 patients worldwide, with 60-120 of these from Singapore.

3.2. Criteria for Recruitment

Potential study participants will be identified on the basis of positive blood cultures by liaison between the investigators and the clinical microbiologists. No “cold-calling” will be performed – the investigator will only approach the patient on the invitation of the treating team (who will have been notified of the blood culture result by the clinical microbiologist). On invitation from the treating team, patients will be approached by a member of the investigating team to evaluate suitability for inclusion (by review of the medical records and discussion with the treating team) and, if appropriate, to obtain informed consent from the patient.

3.3. Inclusion Criteria

- a. Bloodstream infection with *E. coli* or *Klebsiella* spp. with proven non-susceptibility to third generation cephalosporins and susceptibility to meropenem and piperacillin-tazobactam from at least one blood culture draw. This will be determined in accordance with laboratory methods and susceptibility breakpoints defined by EUCAST standards (www.eucast.org). Bacterial identification to species level will be performed using standard laboratory methods (e.g. MALDI-TOF; Bruker) and susceptibility testing using the Vitek2 (BioMerieux) instrument.
- b. No more than 72 hours has elapsed since the first positive blood culture collection.
- c. Patient is aged 21 years and over, and is not pregnant (confirmed by negative pregnancy test in women of childbearing age).
- d. The patient or approved proxy is able to provide informed consent.

3.4. Exclusion Criteria

- a. Patient not expected to survive more than 4 days
- b. Patient allergic to a penicillin or a carbapenem
- c. Patient with significant polymicrobial bacteraemia (that is, a Gram positive skin contaminant

- in one set of blood cultures is not regarded as significant polymicrobial bacteraemia).
- d. Treatment is not with the intent to cure the infection (that is, palliative care is an exclusion).
 - e. Pregnancy or breast-feeding.
 - f. Use of concomitant antimicrobials in the first 4 days after enrolment with known activity against Gram-negative bacilli (except trimethoprim/sulfamethoxazole may be continued as *Pneumocystis* prophylaxis).

3.5. Withdrawal Criteria

Reasons for discontinuation might include allergic reactions to either study drug, the need for additional active antibiotics within the first 4 days after enrolment or any reason that the treating team considers appropriate for withdrawal. Equally the patient can withdraw from the trial at any time. It is possible that the trial could be halted early after DMSB review (planned after first 50 patients recruited).

3.6. Subject Replacement

Patients that drop-out from the study will not be replaced.

4. TRIAL SCHEDULE

Time line	Activity / Treatment	Measurements
Day 1 (within 72 hours of first positive blood culture being drawn)	Consent obtained; randomisation; study drug administration by randomised group; vital signs monitoring; study blood cultures collected; 1st dose of study drug given	Vital signs monitoring; study blood cultures collected; collection of demographics, underlying illnesses, baseline laboratory values.
Day 2	Treatment continued, as defined. Dosage adjustments may be necessary depending on renal function.	Study blood cultures repeated (one set); vital signs monitoring
Day 3	As defined above	Study blood cultures repeated (one set); vital signs monitoring
Day 4	As defined above	Vital signs monitoring; study blood cultures (only if prior day's blood cultures still positive)
Day 5	As defined above. Study drug may be switched to oral or once daily IV step-down	Vital signs monitoring; study blood cultures (only if prior day's blood culture still positive); clinical and microbiologic resolution determined.
Day 14	Final permissible day of study drug administration	Duration of study drug administration determined
Day 30		Patient's outcome determined (mortality, relapse of infection and superinfection)

5. STUDY DESIGN

5.1. Summary of Study Design

The study will use a randomised, controlled phase III non-inferiority trial design comparing two

drug regimens (carbapenem vs. carbapenem-sparing) for bloodstream infections caused by third-generation cephalosporin non-susceptible *E. coli* or *Klebsiella* spp. Recruitment in Singapore will be from any adult patient admitted to NUH. Concurrently 7 other tertiary referral hospitals in Australia and New Zealand will also be recruiting to the trial. Blinding will not be performed as the two antibiotics have different pharmacokinetics. Follow-up will be for 30 days post enrolment. Direct patient contact will be brief and last for 5 days only. Recruitment is planned to start in August 2013 and aims to be completed by December 2015.

6. METHODS AND ASSESSMENTS

Primary aim: 30-day mortality will be assessed by clinical record review and direct patient interview/phone consultation, if applicable.

Secondary aims:

(1) **Time to clinical and microbiologic resolution of infection** – defined as number of days from randomisation to resolution of fever (temperature $> 38.0^{\circ}\text{C}$) and leucocytosis (white blood cell count $>12 \times 10^9/\text{L}$) PLUS sterilisation of blood cultures. This endpoint is relevant given that it uses highly objective criteria to determine resolution of infection. Given this is an unblinded study, we sought only to use objective criteria rather than other clinically defined criteria, such as “resolution of symptoms and signs of infection”, which may be subjective in interpretation.

(2) **Clinical and Microbiologic Success** – defined as survival PLUS resolution of fever and leucocytosis PLUS sterilisation of blood cultures. All of these criteria will be assessed on day 4, counted from the day of randomisation (day 1) in order to determine a rapid response from the trial drug.

(3) **Microbiologic resolution of infection** – defined as sterility of blood cultures collected on or before day 4;

(4) **Microbiologic relapse** – defined as growth of the same organism as in the original blood culture after the end of the period of study drug administration but before day 30;

(5) **Superinfection with a carbapenem resistant organism** – defined as growth of a meropenem resistant Gram negative bacillus from any clinical specimen collected from day 4 of study drug administration to day 30. This endpoint is important since one of the purposes of establishing an alternative to carbapenem therapy is to reduce infections with carbapenem resistant organisms.

There are no assessments with procedures that involve audio, video or image recording.

6.1. Randomisation and Blinding

Patients will be randomly assigned to either meropenem or piperacillin-tazobactam in a 1:1 ratio according to a randomisation list prepared in advance. Random sequence will be generated using random permuted blocks of unequal length. The randomisation process will be managed by the Queensland Clinical Trials & Biostatistics Centre (QCTBC) of The University of Queensland.

6.2. Contraception and Pregnancy Testing

For females of childbearing age included in the trial pregnancy testing will be performed with informed consent (if not already done so by the treating team). As the intervention period is short (4-14 days) and occurring during a hospital admission, it is not anticipated that contraceptive advice is relevant.

6.3. Study Visits and Procedures

a. Screening Visits and Procedures

For inclusion the patient must have fulfilled the microbiological requirements as defined in the inclusion criteria. Once the investigating team has been notified of an eligible patient (as determined by the microbiology laboratory) by request from the treating team, a member of the research team will visit the patient at the bedside. The initial screening visit will include a clinical record review, discussion with the treating team and brief patient interview to determine suitability for inclusion. The patient will then have the study explained to them and be offered an opportunity to be recruited. This will only occur after written material has been provided and the patient has had time to consider and ask questions of the study team or the primary treating team. If consent is given, a single set of blood cultures will be taken by the study team. Once randomised, the first dose of the study drug will be administered by ward nursing staff. Demographic, clinical and laboratory data will be entered into the study clinical record form. Recruitment must be achieved within 72 hours of the initial positive blood culture draw.

b. Study Visits and Procedures

Study visits will continue on days 2-5. Daily blood cultures will be drawn up to day 3, or up to day 5 if persistently positive. Cultures drawn after day 5 will be at the treating team's discretion as dictated by clinical need only. Daily recording of clinical parameters will continue until and including day 5 post enrolment. On day 5 the treating team may decide to switch to oral or IV once daily step down therapy (or continue the allocated study drug) according to their clinical judgement.

c. Final Study Visit:

On day 30 patient outcomes will be determined. This will primarily involve a review of all clinical and laboratory records for that period. It may involve a telephone consultation if the patient has been discharged. There will be no requirement for additional hospital visits or tests.

d. Post Study Follow up and Procedures

There is no requirement for post-study follow-up or procedures.

e. Discontinuation Visit and Procedures

Subjects may withdraw voluntarily from participation in the study at any time. If a patient withdraws for any reason, they will not be subjected to further procedures. Participants who discontinue participation in the study will still have mortality assessment at day 30. Patients who

deviate from intervention protocols will, however, continue to have primary and secondary endpoint assessments for intention to treat analysis. Should withdrawal occur as a result of any adverse event from the study drug, appropriate medical care will continue to be provided by the primary treating team.

7. TRIAL MATERIALS

7.1. Trial Product (s)

Both meropenem and piperacillin-tazobactam are licensed in Singapore. They have been widely used in clinical practice for many years and have proven efficacy in a variety of infectious syndromes, with limited toxicity or adverse reactions.

7.2. Storage and Drug Accountability

Both drugs will be stored and administered in accordance with standard pharmacy procedures; they are both routinely available on the hospital formulary.

8. TREATMENT

8.1. Rationale for Selection of Dose

The dosing regimens selected are the standard schedules for serious infections for both agents. For piperacillin-tazobactam, the use of 4.5g given every 6h (as opposed to 8 hourly) has been shown to have a more favourable PK/PD profile for treating Gram-negative organisms where the MIC is at the higher end of the susceptible range or just below the breakpoint.

8.2. Study Drug Formulations

Both will be given by intravenous injection diluted to the treating dose in the appropriate diluent.

8.3. Study Drug Administration

Meropenem 1 gram administered every 8 hours intravenously or piperacillin/tazobactam 4.5 grams administered every 6 hours intravenously. Each dose will be administered over 30 minutes. Dosing adjustments for meropenem and piperacillin/tazobactam will be made in patients with renal dysfunction, according to the following table (creatinine clearance is expressed in mL/minute).

	Meropenem	Piperacillin/tazobactam
Creatinine clearance >50	No change	No change
Creatinine clearance 26-50	1 gram every 12 hours	4.5 grams every 8 hours
Creatinine clearance 10-25	500 mg every 12 hours	4.5 grams every 12 hours
Creatinine clearance <10	500 mg every 24 hours	4.5 grams every 12 hours
Haemodialysis	500 mg every 24 hours and 500mg after each dialysis	2.25 grams every 8 hours and an additional 0.75 grams after each dialysis

Peritoneal dialysis	500mg every 24 hours	2.25 grams every 8 hours
Continuous-renal replacement therapy	1 gram every 12 hours	4.5 grams every 8 hours

8.4. Specific Restrictions / Requirements

Other antimicrobials active against Gram-negative bacilli are excluded in the first 4 days after enrolment, except that trimethoprim/sulfamethoxazole may be continued as *Pneumocystis* prophylaxis.

8.5. Blinding

Not applicable – this is an open-label trial.

8.6. Concomitant therapy

Relevant concomitant prescribed medication will be documented in the clinical record form.

9. SAFETY MEASUREMENTS

9.1. Definitions

UPIRTSO events and serious adverse events are defined below. Events will be reviewed and classified by the site PI or other investigator. Severity will be classified using a standard set of criteria for grading adverse events (Common Terminology Criteria for Adverse Events version 4.03). The relationship of the event to the study drug and whether the event is an expected event or not will be assessed using the listing of adverse effects contained in the summary of product characteristics for the antibiotics used.

Death within 30 days of bloodstream infection is the primary outcome measure. Given the high mortality associated with the underlying infection (on average around 10%), death itself cannot be considered an ‘unanticipated’ event. Rarely, life threatening allergic reactions can occur with the use of any beta-lactam or carbapenem antibiotic. Although rare, they are well described and form part of the risk-benefit calculation for the use of any antibiotic. Other serious adverse events might include haematological abnormalities (e.g. neutropenia), renal toxicity (e.g. interstitial nephritis), diarrhoea (including *Clostridium difficile* infection) or liver function abnormalities. An increased risk of seizure has been reported with high doses of carbapenems and other beta-lactams. All deaths and adverse events will be notified to the PI and the DMSB. This will primarily be, though not limited to, the responsibility of the research assistants. Unforeseen adverse events will be discussed with collaborating investigators at other centres; such information will be reviewed by regular teleconference. If any member of the trial team becomes aware of an unexpected death or serious adverse event at any stage of the trial review period, the PI will be alerted. All deaths and adverse events will be recorded and reported in the final analysis.

9.2. Collecting, Recording and Reporting of “Unanticipated Problems Involving Risk to Subjects or Others” – UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

Any events that are unexpected (in terms of severity or frequency), that can reasonably be attributed to the study drug under study and that may expose other subjects to harm will be reported. UPIRTSO events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

1. Unexpected

In terms of nature, severity or frequency of the problem as described in the study documentation (eg: Protocol, Consent documents etc).

2. Related or possibly related to participation in the research

Possibly related means there is a reasonable possibility that the problem may have been caused by the procedures involved in the research; and

3. Risk of harm

Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for UPIRTSO Events to the NHG DSRB:

1. Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the NHG investigator.
2. Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the NHG investigator.

9.3. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

All SAEs that are unexpected and related to the study drug will be reported to the HAS within 15 calendar days after initial notification to the PI. For fatal or life-threatening cases, the HSA will be notified as soon as possible but no later than 7 calendar days followed by a complete report within 8 additional calendar days.

A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.
- Is a Medically important event.

Medical and scientific judgment will be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event will be reported as serious.

However, since both the study drugs have been in wide clinical use for many years (amounting to millions of cumulative doses), their effects and possible adverse events are well recognised. As such, although adverse events may occur, we would not anticipate that they would be unexpected or widely divergent from established frequencies.

9.4. Safety Monitoring Plan

A DSMB will be established, comprising two independent infectious disease physicians (Dr Jesus Rodriguez-Bano [Seville, Spain] and Dr Yohei Doi [Pittsburgh, USA]) with statistical support provided to them by the University of Queensland's Queensland Clinical Trials and Biostatistics Centre. An interim analysis – including both efficacy and safety endpoints - will be performed after the first 50 subjects have completed the 30-day study period. The trial statistician will provide details of safety outcomes and any significant differences in primary outcomes according to treatment arm to the DSMB. The stopping rule would be a statistically significant difference in primary outcomes between the two therapies. The interim analysis will be communicated to the local trial team as well as international collaborators along with the DSMB recommendations for action. If there is a significant safety concern raised, the DSMB may recommend to the Principal Investigator that the trial should be stopped

9.5. Complaint Handling

Complaints may be made to the PI or the DSRB. Complaints will be handled according to the normal procedures in operation in NUH.

10. DATA ANALYSIS

10.1. Data Quality Assurance

A random sample of clinical record forms will be regularly double-checked by a senior member of the study team to ensure data quality and accuracy.

10.2. Data Entry and Storage

A clinical database using the FDA approved OpenClinica® trial data management system will be developed with a web hosting facility. Electronic case report forms (eCRFs) will be developed and validated to collect all efficacy, safety and additional laboratory related information. The trial database will include information on demographics (age, gender), underlying illnesses, baseline and follow-up laboratory data including microbiologic data (e.g., organism type, mechanism of resistance and minimal inhibitory concentration (MIC) of study drug), and daily assessments of vital signs and white blood cell counts for the purpose of assessment of clinical outcome. All data queries and corrections will be jointly conducted by the Queensland Clinical Trials & Biostatistics Centre (QCTBC) and the study team prior to database lock. The QCTBC will manage the data and will conduct quality control of the data

following their own standard operating procedures. All analyses performed, the Clinical Study Report(s) and the final data set will be archived together according to QCTBC standard operating procedures and the guidelines of The University of Queensland.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

As no randomized clinical trial has yet been conducted in this particular field, the power analysis for our study is based on the observed 30-day mortality rate reported from the study of bloodstream infection with ESBL producing *E. coli* performed by Rodriguez-Bano et al. The overall 30-day mortality in their study was 16.7% in those patients who received a carbapenem (our control group). We have conducted a series of simulations with possible variations in the observed rates between the two treatment groups.

Considering a mortality rate of 17% in the control group (rounded from the 16.7% actually observed by Rodriguez-Bano), and a non-inferiority margin of 5% difference in the two groups, we need 225 patients in total to achieve 80% power with an alpha level of 0.025. This allows for 10% dropout. It is likely that mortality rates in observational cohorts may be greater than those in an RCT which includes exclusion criteria. Therefore, if the observed mortality rate in the control group was 14% (3% lower than that seen in the observational cohort), then under the same assumptions, we need 310 patients in total to draw inference with 80% power. Thus, our desired sample size is 310 patients in total.

11.2. Statistical and Analytical Plans

a. General Considerations

The intention-to-treat (ITT) analysis approach, supported by the per-protocol approach, will be adopted to make inference on the possible non-inferiority of the treatment arm, compared to the control arm, in terms 30-day mortality. The proportions of deaths along with the confidence intervals of the proportions of deaths in the two study arms will be calculated. Standard statistical inference technique will be employed to draw inference on the possible non-inferiority of the intervention treatment compared to the control treatment. Appropriate parametric or non-parametric statistical techniques will be employed to analyse the data for secondary aims of the study. All secondary analyses will be based on ITT population.

b. Safety Analyses

A Data Safety & Monitoring Board (DSMB) will be constituted by the QCTBC of the University of Queensland to oversee the safety aspect of the study and conduct and interim analysis after 50 patients have completed the 30-day study period.

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

Potential study participants will be identified on the basis of positive blood cultures by liaison between the investigators and the clinical microbiologists. The investigators will only approach the patient on the invitation of the treating team. As soon as practically possible after this discussion, the study team representative will approach the patient at the bedside. Typically this will be around 48-72 hours after the onset of clinical sepsis and the initial collection of blood cultures. Only the PI, or co-investigators will be allowed to obtain informed consent from subjects. Delegated research assistants can perform the initial screening. All will have received appropriate training (including CITI certification). Patients will be given adequate time to consider their options. Although patients may still be unwell at the time of recruitment, informed consent will only be obtained if it is judged that the patient has capacity to make an informed choice. It will be made clear to patients that the study team are not in overall control of their clinical care, which will in no way be affected by their refusal to participate. The person taking consent will not exert undue influence or coerce potential recruits - this will be reinforced to team members by the PI and co-investigators. Patients will be given every opportunity to reverse their decision to enrol in the study. Consent forms will be made available for non-English speakers, including translations in Mandarin, Malay and Tamil. For non-literate subjects, an impartial witness will be asked to certify in writing that the study has been explained in language that the subject understands and that he/she has agreed to participate in the study.

12.2. IRB review

All relevant documents will be made available to the NHG DSRB for review.

12.3. Confidentiality of Data and Patient Records

All study findings and documents will be regarded as confidential. The investigators and other study personnel must not disclose such information without prior written approval from the Principal Investigator. Subject confidentiality will be strictly maintained to the extent possible under the law and as required by Singapore Guideline for Good Clinical Practice (SGGCP). Identifiable information will be removed from any published data.

13. PUBLICATIONS

The data obtained from all participating sites will be pooled and analysed together as soon as possible after trial completion. Individual researchers will not publish data from the trial until the main study publication has been released.

14. RETENTION OF TRIAL DOCUMENTS

Any electronic data records stored locally will be kept only on a single computer located within the Department of Infectious Diseases, within a password-protected folder. The PI will keep any paper-based records, DSRB files or source documentation in a locked cabinet within the department. These records, electronic and physical, will be kept for a minimum of 6 years after

the completion of the trial before being destroyed or erased, as per SGGCP. These documents will be retained for a longer period if required by the applicable regulatory requirements or institutional policy.

Final Trial Protocol

STUDY PROTOCOL

PROTOCOL TITLE:

Randomised Controlled Trial of meropenem versus piperacillin-tazobactam for definitive treatment of bloodstream infections due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* spp. (MERINO Trial)

PROTOCOL VERSION: 6**PROTOCOL DATE:** 26th March 2015**PRINCIPAL INVESTIGATOR:**

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STUDY PROTOCOL

1. BACKGROUND AND RATIONALE

1.1. General Introduction

Escherichia coli and *Klebsiella* spp. are common causes of bacteraemia, and may acquire genes encoding extended-spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases (1). ESBL or AmpC producers are typically resistant to third generation cephalosporins such as ceftriaxone, but susceptible to carbapenems (1). Observational studies have been performed evaluating antibiotic choices for ESBL producers (2-9). In no study has the outcome of treatment for serious infections for ESBL producers been significantly surpassed by carbapenems (2-9).

Despite the potential advantages of carbapenems for treatment of ceftriaxone non-susceptible organisms, widespread use of carbapenems may cause selection pressure leading to carbapenem-resistant organisms. This is a significant issue since carbapenem-resistant organisms are treated with “last-line” antibiotics such as colistin. Some new beta-lactam antibiotics and beta-lactamase inhibitors, which are active against ESBL, AmpC and some carbapenemase producing organisms, are in advanced clinical development (10). However, these antibiotics are likely to be expensive and may best be held in reserve for infections where there are no alternatives. Therefore, we see a need for establishing the efficacy of a generically available alternative to carbapenems for serious infections.

The susceptibility of ESBL producers and AmpC producers to piperacillin/tazobactam is less predictable than that of carbapenems. By definition, ESBLs are inhibited by beta-lactamase inhibitors such as tazobactam (1). However, *E. coli* or *Klebsiella* may produce multiple beta-lactamase types some of which are resistant to inhibition by tazobactam. Additionally, in some cases outer membrane protein loss may contribute to resistance to tazobactam. By definition, AmpC is not inhibited by beta-lactamase inhibitors such as tazobactam. However, despite these limitations, approximately 50% or more of ceftriaxone non-susceptible *E. coli* or *Klebsiellae* remain susceptible in vitro to piperacillin/tazobactam (1).

No randomised controlled trials have yet been performed comparing different treatment options for ceftriaxone resistant Enterobacteriaceae. The largest observational study with an analysis by treatment outcome was published in February 2012 by Rodriguez-Bano and colleagues (9). They performed a post-hoc analysis of six published cohorts of patients with bacteraemia due to ESBL producing *E. coli*. Two nonmutually exclusive cohorts (empirical therapy and definitive therapy) were constructed and analysed separately. In both cohorts, carbapenems were not superior to beta-lactam/beta-lactamase inhibitor combinations (BLBLIC). Specifically, in the definitive therapy cohort, mortality rates at 30 days were not significantly different – 9.3% for those who received a BLBLIC and 16.7% for those who

received a carbapenem ($p > 0.20$) (9).

1.2. Rationale and justification for the Study

Our hypothesis is that piperacillin/tazobactam is non-inferior to meropenem for the definitive treatment of bloodstream infections due to third-generation cephalosporin non-susceptible *E. coli* or *Klebsiella* spp.

The study design will be a randomised controlled multicentre trial. Both study drugs (meropenem and piperacillin/tazobactam) will administered intravenously with standard optimised dosing regimens. The study population will be all adult patients (18 years of age or older) admitted to any ward. Inclusion in the study will be determined by the presence of a bloodstream infection with *E. coli* or *Klebsiella* spp., as defined by at least one positive blood culture from a peripheral blood draw, where the isolate is confirmed to be 3rd generation cephalosporin non-susceptible, but susceptible to piperacillin/tazobactam and meropenem

Meropenem 1 gram will be administered every 8 hours intravenously or piperacillin/tazobactam 4.5 grams administered every 6 hours intravenously. Each dose will be given over 30 minutes. The study drug is to be administered for a minimum of 4 days and can be given for as long as 14 days. The duration of therapy will be determined by the treating clinician.

a. Rationale for the Study Purpose

No randomised controlled trials (RCTs) have yet been performed comparing different treatment options for AmpC or ESBL-producing *Enterobacteriaceae*. During the last 10 years we have seen an exponentially increasing rate of carbapenem resistance worldwide, including Australia and New Zealand. We urgently need data from well-designed RCTs to guide clinicians in the treatment of antibiotic resistant Gram-negative infections. We face a situation where a commonly used antibiotic for these infections (meropenem) may be driving carbapenem resistance. For this reason, we are seeking to compare a carbapenem-sparing regimen with a carbapenem for the treatment of these infections. Formal evaluation of safety and efficacy of generic antibiotics in the treatment of infection is of immense clinical and public health importance, and no formal trial has yet been conducted to address these issues. The international collaboration between teams of clinician researchers, some of whom are leaders in their field, makes it highly likely that the outcomes of this trial will have a significant impact on clinical practice.

b. Rationale for Doses Selected

The dosing regimens selected are the standard schedules for serious infections for both agents. For piperacillin/tazobactam, the use of 4.5g given every 6h (as opposed to 8 hourly) has been shown to have a more favourable pharmacokinetic/pharmacodynamic (PK/PD) profile for treating Gram-negative organisms where the MIC is at the higher end of the susceptible range or just below the breakpoint.

c. Rationale for Study Population

The aim of the study is to help clinicians and microbiologists make evidence-based therapeutic decisions for patients with serious infections caused by ESBL producers. As ESBL infections

can be seen in a large range of clinical situations, spanning all departments and patient populations, the study logically requires recruitment of patients from all areas of the hospital system. In general, these infections are usually treated in admitted patients (due to their severity), and so inclusion of out-patients is not applicable.

d. Rationale for Study Design

The optimal study design to answer whether one drug (piperacillin/tazobactam) is non-inferior in comparison to another (meropenem) is a randomised controlled trial (RCT). To date, no RCTs have yet been performed in this specific area. The common recommendation for the use of carbapenems is based on non-randomised observational studies, which always have the inherent problems of bias and confounding factors. Such methodological issues always weaken interpretations derived from these studies. A well-conducted RCT will provide the highest possible level of evidence upon which to base clinical decision-making.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

Our hypothesis is that piperacillin/tazobactam (a carbapenem-sparing regimen) is non-inferior to meropenem (a widely used carbapenem) for the definitive treatment of bloodstream infections due to third-generation cephalosporin non-susceptible *E. coli* or *Klebsiella* spp.

2.2. Primary Objectives

To compare the 30-day mortality post bloodstream infection of piperacillin/tazobactam and meropenem.

2.3. Secondary Objectives

- (1) To compare the time to clinical and microbiologic resolution of infection for each regimen;
- (2) To compare the clinical and microbiologic success of each regimen at day 4 of the intervention;
- (3) To compare the risk of relapse with each regimen;
- (4) To compare the risk of superinfection with a carbapenem resistant organism with each regimen

2.4. Potential Risks and benefits:

a. End Points - Efficacy

Retrospective studies suggest that piperacillin/tazobactam is non-inferior to meropenem in the treatment of ESBL-producing bacteria. Therefore, we do not anticipate that either drug will provide specific benefits or additional efficacy. However, piperacillin/tazobactam may provide less selective pressure for colonisation and infection with carbapenem-resistant bacteria.

b. End Points - Safety

The main risk to the study population is that piperacillin/tazobactam may prove inferior to meropenem in the treatment of ESBL-producers. However, both drugs are currently commonly used in clinical practice for this indication when *in vitro* susceptibility is reported. We therefore, do not believe that we are exposing patients to excess risk by study inclusion beyond the risks involved in standard therapeutic decisions and clinical management.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

The study is a multi-centre study, involving institutions across Australia, New Zealand and Singapore. The study population will be drawn from any patient admitted to hospital with a blood culture positive for third generation cephalosporin non-susceptible *E. coli* or *Klebsiella*. There will be no restrictions on race, gender or ethnicity. Minors (aged <18y) have been excluded both to simplify the consent procedure and since the response to infection in children may be different than in adults (for instance, shorter duration of therapy is commonly used).

3.2. Criteria for Recruitment

Potential study participants will be identified on the basis of positive blood cultures by liaison between the investigators and the clinical microbiologists. No “cold-calling” will be performed – the investigator will only approach the patient on the invitation of the treating team (who will have been notified of the blood culture result by the clinical microbiologist). Only once invitation from the patient and the treating team has been granted, will patients be approached by a member of the investigating team to evaluate suitability for inclusion (by review of the medical records and discussion with the treating team) and, when appropriate, to obtain informed consent from the patient or legal substitute decision maker.

3.3. Inclusion Criteria

- a. Bloodstream infection with *E. coli* or *Klebsiella* spp. with proven non-susceptibility to third generation cephalosporins and susceptibility to meropenem and piperacillin-tazobactam from at least one blood culture draw. This will be determined in accordance with laboratory methods and susceptibility breakpoints defined by EUCAST standards (www.eucast.org). Bacterial identification to species level will be performed using standard laboratory methods (e.g. MALDI-TOF) and susceptibility testing (e.g. Vitek2)
- b. No more than 72 hours has elapsed since the first positive blood culture collection.
- c. Patient is aged 18 years and over
- d. The patient or approved proxy is able to provide informed consent.

3.4. Exclusion Criteria

- a. Patient not expected to survive more than 4 days
- b. Patient allergic to a penicillin or a carbapenem
- c. Patient with significant polymicrobial bacteraemia (that is, a Gram positive skin contaminant in one set of blood cultures is not regarded as significant polymicrobial bacteraemia).
- d. Treatment is not with the intent to cure the infection (that is, palliative care is an exclusion).
- e. Pregnancy or breast-feeding.

f. Use of concomitant antimicrobials in the first 4 days after enrolment with known activity against Gram-negative bacilli (except trimethoprim/sulfamethoxazole may be continued as *Pneumocystis* prophylaxis).

3.5. Withdrawal Criteria

Reasons for discontinuation might include allergic reactions to either study drug, the need for additional active antibiotics within the first 4 days after enrolment or any reason that the treating team considers appropriate for withdrawal. Equally the patient can withdraw from the trial at any time. It is possible that the trial could be halted early after DMSB review (planned after first 50 patients recruited).

3.6. Subject Replacement

Patients that drop-out from the study will not be replaced.

4. TRIAL SCHEDULE

Time line	Activity / Treatment	Measurements
Day 1 (within 72 hours of first positive blood culture being drawn)	Consent obtained; randomisation; study drug administration by randomised group; vital signs monitoring; study blood cultures collected; 1st dose of study drug given	Vital signs monitoring; study blood cultures collected if febrile (temp >38°C); collection of demographics, underlying illnesses, baseline laboratory values.
Day 2	Treatment continued, as defined. Dosage adjustments may be necessary depending on renal function.	Study blood cultures repeated (one set) only if febrile >38°C previous 24h or if previous day BC positive; vital signs monitoring
Day 3	As defined above	Study blood cultures repeated (one set) in all patients (to document clearance); vital signs monitoring
Day 4	As defined above	Vital signs monitoring; study blood cultures (only if prior day's blood cultures still positive or if febrile)
Day 5	As defined above. Study drug may be switched to oral or once daily IV step-down	Vital signs monitoring; study blood cultures (only if prior day's blood culture still positive or if febrile); clinical and microbiologic resolution determined.
Day 14	Final permissible day of study drug administration	Duration of study drug administration determined
Day 30		Patient's outcome determined (mortality, relapse of infection and superinfection)
Daily FBC to be collected (if not collected as part of routine clinical care) until WCC <12 x10 ⁹ /L		

5. STUDY DESIGN

5.1. Summary of Study Design

The study will use a randomised, controlled phase III non-inferiority trial design comparing two drug regimens (carbapenem vs. carbapenem-sparing) for bloodstream infections caused by third-generation cephalosporin non-susceptible *E. coli* or *Klebsiella* spp. Blinding will not be performed as the two antibiotics have different pharmacokinetics. Follow-up will be for 30 days post enrolment. Direct patient contact will be brief and last for 5 days only.

6. METHODS AND ASSESSMENTS

Primary aim: 30-day mortality will be assessed by clinical record review and direct patient interview/phone consultation, if applicable.

Secondary aims:

- (1) **Time to clinical and microbiologic resolution of infection** – defined as number of days from randomisation to resolution of fever (temperature $> 38.0^{\circ}\text{C}$) and leucocytosis (white blood cell count $>12 \times 10^9/\text{L}$) PLUS sterilisation of blood cultures. This endpoint is relevant given that it uses highly objective criteria to determine resolution of infection. Given this is an unblinded study, we sought only to use objective criteria rather than other clinically defined criteria, such as “resolution of symptoms and signs of infection”, which may be subjective in interpretation.
- (2) **Clinical and Microbiologic Success** – defined as survival PLUS resolution of fever and leucocytosis PLUS sterilisation of blood cultures. All of these criteria will be assessed on day 4, counted from the day of randomisation (day 1) in order to determine a rapid response from the trial drug.
- (3) **Microbiologic resolution of infection** – defined as sterility of blood cultures collected on or before day 4;
- (4) **Microbiologic relapse** – defined as growth of the same organism as in the original blood culture after the end of the period of study drug administration but before day 30;
- (5) **Superinfection with a carbapenem or piperacillin-tazobactam resistant organism or *Clostridium difficile*** – defined as growth of a meropenem resistant Gram negative bacillus from any clinical specimen collected or a positive stool test (according to local lab diagnostic procedures) for *C. difficile*, from day 4 of study drug administration to day 30. This endpoint is important since one of the purposes of establishing an alternative to carbapenem therapy is to reduce infections with resistant organisms.

There are no assessments with procedures that involve audio, video or image recording.

6.1. Randomisation and Blinding

Patients will be randomly assigned to either meropenem or piperacillin-tazobactam in a 1:1 ratio according to a randomisation list prepared in advance. Random sequence will be generated using random permuted blocks of unequal length. The randomisation process will be managed by the Queensland Clinical Trials & Biostatistics Centre (QCTBC) of The University of Queensland via an online module within the REDCap data management system.

6.2. Study Visits and Procedures

a. Screening Visits and Procedures

For inclusion the patient must have fulfilled the microbiological requirements as defined in the inclusion criteria. Once the investigating team has been notified of an eligible patient (as determined by the microbiology laboratory) by request from the treating team, a member of the research team will visit the patient at the bedside. The initial screening visit will include a clinical record review, discussion with the treating team and brief patient interview to determine suitability for inclusion. The patient will then have the study explained to them and be offered an opportunity to be recruited. This will only occur after written material has been provided and the patient has had time to consider and ask questions of the study team or the primary treating team. If consent is given, a single set of blood cultures will be taken by the study team. Once randomised, the first dose of the study drug will be administered by ward nursing staff. Demographic, clinical and laboratory data will be entered into the study clinical record form. Recruitment must be achieved within 72 hours of the initial positive blood culture draw.

b. Study Visits and Procedures

Study visits will continue on days 2-5. Blood cultures will be drawn on day 3, or on other days up to day 5 if the patient is febrile (temp $>38^{\circ}\text{C}$) or if previous day blood culture is positive. Cultures drawn after day 5 will be at the treating team's discretion as dictated by clinical need only. Daily recording of clinical parameters will continue until and including day 5 post enrolment. On day 5 the treating team may decide to switch to oral or IV once daily step down therapy (or continue the allocated study drug) according to their clinical judgement.

c. Final Study Visit:

On day 30 patient outcomes will be determined. This will primarily involve a review of all clinical and laboratory records for that period. It may involve a telephone consultation if the patient has been discharged. There will be no requirement for additional hospital visits or tests.

d. Post Study Follow up and Procedures

There is no requirement for post-study follow-up or procedures.

e. Discontinuation Visit and Procedures

Subjects may withdraw voluntarily from participation in the study at any time. If a patient withdraws for any reason, they will not be subjected to further procedures. Participants who discontinue participation in the study will still have mortality assessment at day 30. Patients who deviate from intervention protocols will, however, continue to have primary and secondary endpoint assessments for intention to treat analysis. Should withdrawal occur as a result of any adverse event from the study drug, appropriate medical care will continue to be provided by the primary treating team.

7. TRIAL MATERIALS

7.1. Trial Product (s)

Both meropenem and piperacillin-tazobactam are licensed in Australia, Singapore and New Zealand. They have been widely used in clinical practice for many years and have proven efficacy in a variety of infectious syndromes, with limited toxicity or adverse reactions.

7.2. Storage and Drug Accountability

Both drugs will be stored and administered in accordance with standard pharmacy procedures; they are both routinely available on the hospital formulary.

8. TREATMENT

8.1. Rationale for Selection of Dose

The dosing regimens selected are the standard schedules for serious infections for both agents. For piperacillin-tazobactam, the use of 4.5g given every 6h (as opposed to 8 hourly) has been shown to have a more favourable PK/PD profile for treating Gram-negative organisms where the MIC is at the higher end of the susceptible range or just below the breakpoint.

8.2. Study Drug Formulations

Both will be given by intravenous injection diluted to the treating dose in the appropriate diluent.

8.3. Study Drug Administration

Meropenem 1 gram administered every 8 hours intravenously or piperacillin/tazobactam 4.5 grams administered every 6 hours intravenously. Each dose will be administered over 30 minutes. Dosing adjustments for meropenem and piperacillin/tazobactam will be made in patients with renal dysfunction, according to the following table (creatinine clearance is expressed in mL/minute).

	Meropenem	Piperacillin/tazobactam
Creatinine clearance >50	No change	No change
Creatinine clearance 26-50	1 gram every 12 hours	4.5 grams every 8 hours
Creatinine clearance 10-25	500 mg every 12 hours	4.5 grams every 12 hours
Creatinine clearance <10	500 mg every 24 hours	4.5 grams every 12 hours
Haemodialysis	500 mg every 24 hours and 500mg after each dialysis	2.25 grams every 8 hours and an additional 0.75 grams after each dialysis
Peritoneal dialysis	500mg every 24 hours	2.25 grams every 8 hours
Continuous-renal replacement therapy	1 gram every 12 hours	4.5 grams every 8 hours

8.4. Specific Restrictions / Requirements

Other antimicrobials active against Gram-negative bacilli are excluded in the first 4 days after enrolment, except that trimethoprim/sulfamethoxazole may be continued as *Pneumocystis* prophylaxis.

8.5. Blinding

Not applicable – this is an open-label trial.

8.6. Concomitant therapy

Relevant concomitant prescribed medication will be documented in the clinical record form.

9. SAFETY MEASUREMENTS

9.1. Definitions

An adverse event is defined in the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice as “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.”

Events will be reviewed and classified by the site PI. The relationship of the event to the study drug and whether the event is an expected event or not will be assessed using the listing of adverse effects contained in the summary of product characteristics for the antibiotics used.

The treating team has the primary responsibility for reviewing laboratory test results and determining whether an abnormal value in an individual study participant requires action. In general, abnormal laboratory without clinical significance (based on clinical judgment) should not be recorded as adverse events; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered adverse. The investigators should liaise closely with the treating teams and remain aware of any such adverse events.

Serious adverse event (SAE) are defined as an adverse event that:

- is fatal
- is life threatening (places the participant at immediate risk of death)
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- other significant medical hazard

However, since both the study drugs have been in wide clinical use for many years (amounting to millions of cumulative doses), their effects and possible adverse events are well recognised. As such, although adverse events may occur, we would not anticipate that they would be unexpected or widely divergent from established frequencies.

Death within 30 days of bloodstream infection is the primary outcome measure. Given the high mortality associated with the underlying infection (on average around 10%), death itself cannot be considered an ‘unanticipated’ event. Rarely, life threatening allergic reactions can occur with the use of any beta-lactam or carbapenem antibiotic. Although rare, they are well described and form part of the risk-benefit calculation for the use of any antibiotic. Other serious adverse events might include haematological abnormalities (e.g. neutropenia), renal toxicity (e.g. interstitial nephritis), diarrhoea (including *Clostridium difficile* infection) or liver function abnormalities. An increased risk of seizure has been reported with high doses of carbapenems and other beta-lactams.

All deaths and SAEs will be notified to the local PI and site research governance, along with the trial management team at UQCCR, who will then notify the RBWH HREC. Unforeseen adverse events will be discussed with collaborating investigators at other centres; such information will be reviewed by regular teleconference. If any member of the trial team becomes aware of an unexpected death or serious adverse event at any stage of the trial review period, the PI will be alerted. All deaths and adverse events will be recorded and reported in the final analysis.

9.2. Collecting, Recording and Reporting of “Serious Adverse Event (SAE)/Safety/Suspected Unexpected Serious Adverse Reaction (SUSAR)”

Any events that are unexpected (in terms of severity or frequency), that can reasonably be attributed to the drug under study and that may expose other subjects to harm will be reported. SAE/Safety/SUSAR events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

Unexpected

In terms of nature, severity or frequency of the problem as described in the study documentation (e.g.: Protocol, Consent documents etc.).

Related or possibly related to participation in the research

Possibly related means there is a reasonable possibility that the problem may have been caused by the procedures involved in the research; and

Risk of harm

Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for SAE/Safety/SUSAR Events:

Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the local PI.

Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the local investigator.

9.3. Safety Monitoring Plan

A DSMB will be established, comprising two independent infectious disease physicians (Dr. Jesus Rodriguez-Bano [Seville, Spain] and Dr. Yohei Doi [Pittsburgh, USA]) with statistical support provided to them by the University of Queensland's Queensland Clinical Trials and Biostatistics Centre. An interim analysis – including both efficacy and safety endpoints - will be performed after the first 50 subjects have completed the 30-day study period. The trial statistician will provide details of safety outcomes and any significant differences in primary outcomes according to treatment arm to the DSMB. The stopping rule would be a statistically significant difference in primary outcomes between the two therapies (using the Peto rule for interim analyses, $p < 0.001$). The interim analysis will be communicated to the local trial team as well as all national and international collaborators along with the DSMB recommendations for action. If there is a significant safety concern raised, the DSMB may recommend to the Principal Investigator that the trial should be stopped. The timing of additional interim analyses will be determined by the DSMB.

9.4. Complaint Handling

Complaints may be made to the PI or approving HREC. Complaints will be handled according to the normal procedures in operation at the recruiting hospital.

10. DATA ANALYSIS

10.1. Data Quality Assurance

A random sample of clinical record forms will be regularly double-checked by a senior member of the study team to ensure data quality and accuracy.

10.2. Data Entry and Storage

A clinical database using the REDCap trial data management system has been developed with a web hosting facility. Electronic case report forms (eCRFs) have been developed and validated to collect all clinical and laboratory related information. The trial database will include information on demographics (age, gender), underlying illnesses, baseline and follow-up laboratory data including microbiologic data (e.g., organism type, mechanism of resistance and minimal inhibitory concentration (MIC) of study drug), and daily assessments of vital signs and white blood cell counts for the purpose of assessment of clinical outcome. All data queries and corrections will be jointly conducted by the Queensland Clinical Trials & Biostatistics Centre (QCTBC) and the study team prior to database lock. The QCTBC will manage the data and will conduct quality control of the data following their own standard operating procedures. All analyses performed, the Clinical Study Report(s) and the final data set will be archived together according to QCTBC standard operating procedures and the guidelines of The University of Queensland.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

As no randomised clinical trial has yet been conducted in this particular field, the sample size estimation has been derived from the retrospective study of bloodstream infection caused by ESBL-producing *E. coli* performed by Rodriguez-Bano et al. The overall 30-day mortality in their study was 16.7% in those patients who received a carbapenem (our control group). We have conducted a series of simulations with possible variations in the observed rates between the two treatment groups. Considering a mortality rate of 17% in the control group (rounded from the 16.7% actually observed), and a non-inferiority margin of 5% difference in the 2 groups, we would need 280 patients in total to achieve 80% power with a 1-sided alpha level of 0.025. This allows for 10% dropout. It is likely that mortality rates in observational cohorts may be greater than those in a trial with exclusion criteria. Therefore, if the observed mortality rate in the control group was 14% (3% lower than that seen in the observational cohort), then under the same assumptions, we would need 454 patients in total to achieve 80% power

11.2. Statistical and Analytical Plans

a. General Considerations

The intention-to-treat (ITT) analysis approach, supported by the per-protocol approach, will be adopted to make inference on the possible non-inferiority of the treatment arm, compared to the control arm, in terms 30-day mortality. The proportions of deaths along with the confidence intervals of the proportions of deaths in the two study arms will be calculated. Standard statistical inference technique will be employed to draw inference on the possible non-inferiority of the intervention treatment compared to the control treatment. Appropriate parametric or non-parametric statistical techniques will be employed to analyse the data for secondary aims of the study. All secondary analyses will be based on ITT population.

b. Safety Analyses

A Data Safety & Monitoring Board (DSMB) will be constituted by the QCTBC of the University of Queensland to oversee the safety aspect of the study and conduct and interim analysis after 50 patients have completed the 30-day study period.

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

Potential study participants will be identified on the basis of positive blood cultures by liaison between the investigators and the clinical microbiologists. The investigators will only approach the patient on the invitation of the treating team. As soon as practically possible after this discussion, the study team representative will approach the patient at the bedside. Typically this will be around 48-72 hours after the onset of clinical sepsis and the initial collection of blood cultures. Only the PI, or co-investigators will be allowed to obtain informed consent from subjects. Delegated research assistants can perform the initial screening. All will have received appropriate training. Patients will be given adequate time to consider their options. Although

patients may still be unwell at the time of recruitment, informed consent will only be obtained if it is judged that the patient has capacity to make an informed choice. It will be made clear to patients that the study team are not in overall control of their clinical care, which will in no way be affected by their refusal to participate. The person taking consent will not exert undue influence or coerce potential recruits - this will be reinforced to team members by the PI and co-investigators. Patients will be given every opportunity to reverse their decision to enrol in the study. For non-English speakers, qualified translators will be provided as per local hospital protocols. For non-literate subjects, an impartial witness will be asked to certify in writing that the study has been explained in language that the subject understands and that he/she has agreed to participate in the study. For cognitively-impaired patients a legally appropriate proxy should be approached to provide consent to participate. Should the patient regain cognition, (s)he should be informed of the previous discussion and have the trial explained to them. Patients should be offered the chance to receive a written summary of the trial after completion and publication should they wish – if so, a record of the contact details will be kept for this purpose.

12.2. IRB review

All relevant documents will be made available to the RBWH HREC for review.

12.3. Confidentiality of Data and Patient Records

All study findings and documents will be regarded as confidential. The investigators and other study personnel must not disclose such information without prior written approval from the Principal Investigator. Subject confidentiality will be strictly maintained to the extent possible under the law and local hospital policy. Identifiable information will be removed from any published data.

13. PUBLICATIONS

The data obtained from all participating sites will be pooled and analysed together as soon as possible after trial completion. Individual researchers will not publish data from the trial until the main study publication has been released.

14. RETENTION OF TRIAL DOCUMENTS

Any electronic data records stored locally will be kept only on a single computer located within the relevant department, using a password-protected folder. The PI will keep any paper-based records, study files or source documentation in a locked cabinet within the department. These records, electronic and physical, will be kept for a minimum of 15 years after the completion of the trial before being destroyed or erased, as per NHMRC guidelines. These documents will be retained for a longer period if required by the applicable regulatory requirements or institutional policy.

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Summary of Protocol changes

The study protocol was published in BMC Trials in Jan 2015 and can be accessed here: <https://www.ncbi.nlm.nih.gov/pubmed/25623485>

Date	Summary of changes
8 th May 2013	Trial Protocol Finalised
11 th Feb 2014	First Patient enrolled (Singapore)
7 th April 2014	Addition of Australian recruiting sites, site investigators updated
18 th June 2014	Amendments <ul style="list-style-type: none"> • Age ≥ 18 years as inclusion (for non-Singapore sites where age of majority is 21 years) • Secondary outcome 5 amended to “Superinfection with a carbapenem or piperacillin-tazobactam resistant organism or <i>Clostridium difficile</i>” (i.e. addition of piperacillin-tazobactam resistance and <i>C. difficile</i>) • Data management system changed from OpenClinica to REDCap • Sample size re-calculated (reconsidered by new trial statistician); revised to 454 patients • Addition of details for SAE / “SUSAR” definitions and reporting to align with Australian NHMRC guidelines
21 st Sept 2014	Trial protocol submitted for publication BMC Trials (published online 27 th Jan 2015)
26 th March 2015 (Final protocol version)	<ul style="list-style-type: none"> • Removal of requirement for daily blood cultures for first 3 days post randomization. <ul style="list-style-type: none"> ○ Changed to: “<i>Blood cultures will be drawn on day 3, or on other days up to day 5 if the patient is febrile (temp >38°C) or if previous day blood culture is positive</i>” • Addition in trial schedule of stipulation to collect daily FBC until white cell count $\leq 12 \times 10^9/L$ • Further details provided on the statistical ruling for interim analyses, along with the interval timing of DSMB reviews and clarification of stopping rules (Peto rule)

Original Statistical Analysis Plan

**Randomised Controlled Trial of Meropenem versus Piperacillin-
Tazobactam for Definitive Treatment of Bloodstream Infections
Due to Ceftriaxone Non-Susceptible
Escherichia coli and Klebsiella spp.
(*The “MERINO trial”*)**

1 INTRODUCTION

This document describes the Statistical Analysis Plan (SAP) for the “MERINO” trial, a multicentre, randomised clinical trial of meropenem compared with piperacillin/tazobactam for definitive therapy of bloodstream infection due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* species. It details the statistical methods to be used and outlines the planned analyses for the main study. This SAP was developed before the initiation of the study.

1.1 Study Objective

This trial is conducted to evaluate if piperacillin/tazobactam was non-inferior to meropenem when used as definitive therapy bloodstream infection due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* species.

1.2 Primary Study Objective

Definition of Study Endpoints: Study endpoints are defined based on clinical standards and regulatory precedent. The primary aim of this study is to compare the effects of two antibiotics on mortality following bloodstream infection up to day 30, with day 1 defined as the date of randomisation

Hypothesis: The administration of piperacillin/tazobactam is non-inferior to meropenem with respect to mortality following bloodstream infection due to ceftriaxone non-susceptible *E. coli* or *Klebsiella*.

1.3 Secondary Study Objectives

To compare the effect of meropenem and piperacillin/tazobactam on:

- (1) Time to clinical and microbiologic resolution of infection – defined as number of days from *randomisation* to resolution of fever (temperature > 38.0° C) PLUS sterilisation of blood cultures.
- (2) Clinical and Microbiologic Success – defined as survival PLUS resolution of fever and leucocytosis PLUS sterilisation of blood cultures. All of these criteria will be assessed on day 4, counted from the day of randomisation (day 1) in order to determine a rapid response from the trial drug.

- (3) Microbiologic resolution of infection – defined as sterility of blood cultures collected on or before day 4
- (4) Microbiologic relapse – defined as growth of the same organism as in the original blood culture after the end of the period of study drug administration but before day 30
- (5) Superinfection with a carbapenem or piperacillin-tazobactam resistant organism or *Clostridium difficile* - defined as growth of a meropenem or piperacillin-tazobactam resistant organism from any clinical specimen collected from day 4 of study drug administration to day 30 or a positive *C. difficile* stool test. This endpoint is important since one of the purposes of establishing an alternative to carbapenem therapy is to reduce infections with resistant organisms or *C. difficile*.

Interim Analyses

An interim analysis is planned for this trial after 50, 100, 200 and 300 patients are enrolled. A separate data safety and monitoring board is responsible for the safety assessment of the trial during its conduct.

2 ANALYSIS DATA SETS

Analyses will be conducted on the following data sets:

2.1 Intention to Treat (ITT) Analysis Data Set

The ITT data set will include all randomised study participants regardless of their compliance with the rules of the study. The ITT data set will be used for the analyses of all primary and secondary end points. All safety-related analyses will be based on the ITT population.

2.2 Per Protocol (PP) Analysis Data Set

Participants who were randomised but did not receive blinded study medication are excluded from the PP analyses. The PP data set includes participants who were still receiving study medication on day 4.

2.3 Protocol Violations

All protocol violations occurring after randomisation will be listed in the Clinical Study Report (CSR), tabulated by Study Subject ID and study centre. The final assignment of participants to the PP analysis population will be made at a protocol violation review meeting prior to database lock.

3 STATISTICAL METHODOLOGY

The three major sets of analyses to be performed are:

- (1) Primary efficacy analysis: Statistical analytic strategy relating to the primary hypothesis.
- (2) Analysis of secondary efficacy endpoints: Statistical analytic strategy relating to the secondary efficacy endpoints.

The statistical methodologies for each set of analyses are described below, unless noted otherwise.

3.1 Data Export and Archive

The study database is maintained at the Queensland Clinical Trials & Biostatistics Centre (QCTBC) of The University of Queensland. All data queries and corrections will be jointly conducted by the QCTBC and the MERINO study team prior to database lock. The QCTBC will manage the data and will conduct quality control of the data following their own standard operating procedures. All analyses performed, the Clinical Study Report(s) and the final data set will be archived together according to QCTBC standard operating procedures and the guidelines of The University of Queensland.

3.2 Data Validation

Data received by the QCTBC Statistician will be examined for missing values and outliers. Measures of central tendency and dispersion for continuous study parameters will be portrayed along with Box and Whisker plots. Extreme or unexpected values will be examined individually for authenticity and data discrepancies addressed where appropriate. Additional audit and statistical checks will be performed as necessary.

3.3 Imputation of Missing Data

No imputation of missing data will be conducted. Missing data for relevant study parameters, if any, will be presented in accordance with standard procedures.

3.4 Primary Efficacy Endpoint

3.4.1 Comparison of mortality by Day 30

The primary aim of this study is to compare the effects of two antibiotics on mortality on or before day 30. Since this is a non-inferiority study both the ITT population and the PP population will be used for this analysis. Non-inferiority must be met for analyses of both the ITT and PP population for piperacillin/tazobactam to be regarded as non-inferior to meropenem.

Mortality occurring at any time from randomisation up to and including day 30 will be rated as a binary variable (Yes/No). Logistic regression analysis will be conducted with 'treatment group' as the only covariate. The odds ratio along with its 95% confidence interval will be presented with the meropenem arm as the reference group. The proportion of patients [n (%)] who died will be reported for both treatment groups.

3.5 Secondary Efficacy Endpoints

Both the ITT and PP approaches will be used for the analyses of all secondary efficacy endpoints.

4.5.1 Time to clinical and microbiologic resolution of infection – the time to clinical and microbiologic resolution is the 'first' day on which fever is not present and blood cultures are not positive. The median number of days from randomisation to clinical and microbiologic resolution will be compared between the two treatment groups using a Mann-Whitney U test. The time to clinical and microbiologic resolution and 95% confidence interval of the median will be presented.

4.5.2 Clinical and Microbiologic Success – defined as survival at any time on day 4 PLUS resolution of fever on or before day 4 PLUS sterilisation of blood cultures collected on or before day 4

Clinical and microbiologic success at day 4 will be rated as a binary variable (Yes/No). Logistic regression analysis will be conducted with ‘treatment group’ as the only covariate. The odds ratio along with its 95% confidence interval will be presented with the meropenem arm as the reference group. The proportion of patients [n (%)] who have clinical and microbiologic success will be reported for both treatment groups.

4.5.3 Microbiologic resolution of infection – defined as sterility of blood cultures collected on or before day 4

Microbiologic resolution will be rated as a binary variable (Yes/No). Logistic regression analysis will be conducted with ‘treatment group’ as the only covariate. The odds ratio along with its 95% confidence interval will be presented with the meropenem arm as the reference group. The proportion of patients [n (%)] who have microbiologic resolution will be reported for both treatment groups.

4.5.4 Microbiologic relapse – defined as growth of the same organism as in the original blood culture after the end of the period of study drug administration but before 30 days after the first positive blood culture was drawn

Microbiologic relapse will be rated as a binary variable (Yes/No). Logistic regression analysis will be conducted with ‘treatment group’ as the only covariate. The odds ratio along with its 95% confidence interval will be presented with the meropenem arm as the reference group. The proportion of patients [n (%)] who have microbiologic relapse will be reported for both treatment groups.

4.5.5 Superinfection with a carbapenem resistant organism – defined as growth of a meropenem resistant Gram negative bacillus from any clinical specimen collected from the time of randomisation to day 30

Superinfection with a carbapenem resistant organism will be rated as a binary variable (Yes/No). Logistic regression analysis will be conducted with ‘treatment group’ as the only covariate. The odds ratio along with its 95% confidence interval will be presented with the meropenem arm as the reference group. The proportion of patients [n (%)] who have superinfection will be reported for both treatment groups.

4.6 Subgroup analyses

An analysis of the primary and secondary efficacy endpoints as described above is proposed in the following subgroups, which is that of “high risk patients” defined by any of the following:

- (1) likely source of infection other than urinary tract,
- (2) Pitt score ≥ 4 on day 1

The Pitt bacteremia score will be calculated using the following criteria:

- oral temperature: 2 points for a temperature of $\leq 35^{\circ}$ C or $\geq 40^{\circ}$ C, 1 point for a temperature of $35.1\text{--}36.0^{\circ}$ C or $39.0\text{--}39.9^{\circ}$ C, and 0 points for a temperature of $36.1\text{--}38.9^{\circ}$ C;
- hypotension: 2 points for an acute hypotensive event with decreases in systolic and diastolic blood pressure of 130 and 120 mm Hg, respectively, use of intravenous vasopressor agents, or systolic blood pressure <90 mm Hg;
- receipt of mechanical ventilation: 2 points;
- cardiac arrest: 4 points;
- mental status: alert, 0 points; disoriented, 1 point; stuporous, 2 points; and comatose, 4 points.

7 BASIC STATISTICS IN THE CLINICAL STUDY REPORT

The basic statistics specified in the CSR will consist of the following:

- Information on missing values for all relevant study variables
- Summary of baseline categorical study variables with n (%); minimum, maximum, mean, standard deviation and first, second & third quartiles for continuous study variables
- Presentation by sub-categories including treatment allocation and study centre where appropriate
- For continuous study variables, box plots and Kernel density plots will also be provided as appropriate.

Final Statistical Analysis Plan

**Randomised Controlled Trial of Meropenem versus Piperacillin-
Tazobactam for Definitive Treatment of Bloodstream Infections
Due to Ceftriaxone Non-Susceptible
Escherichia coli and Klebsiella spp.
(*The “MERINO trial”*)**

Statistical Analysis Plan

Version 3.1; Date: 26th April 2017

1 INTRODUCTION

This document describes the Statistical Analysis Plan (SAP) for the “MERINO” trial, a multicentre, randomised clinical trial of meropenem compared with piperacillin/tazobactam for definitive therapy of bloodstream infection due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* species. It details the statistical methods to be used and outlines the planned analyses for the main study. This SAP was developed before the initiation of the study, with minor amendments made prior to final analysis following recommendations by the DSMB. The study protocol was published prior to commencement of the trial (Harris *et al* 2015).

1.1 Study Objective

This trial is conducted to evaluate if piperacillin/tazobactam was non-inferior to meropenem when used as definitive therapy bloodstream infection due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* species.

1.2 Primary Study Objective

Definition of Study Endpoints: Study endpoints are defined based on clinical standards and regulatory precedent. The primary aim of this study is to compare the effects of two antibiotics on mortality following bloodstream infection up to day 30, with day 1 defined as the date of randomisation

Hypothesis: The administration of piperacillin/tazobactam is non-inferior to meropenem with respect to mortality following bloodstream infection due to ceftriaxone non-susceptible *E. coli* or *Klebsiella*.

1.3 Secondary Study Objectives

To compare the effect of meropenem and piperacillin/tazobactam on:

- (1) Time to clinical and microbiologic resolution of infection – defined as number of days from *randomisation* to resolution of fever (temperature > 38.0° C) PLUS resolution of leucocytosis (total WCC >12 x10⁹/L) PLUS sterilisation of blood cultures.
- (2) Clinical and Microbiologic Success – defined as survival PLUS resolution of fever and leucocytosis PLUS sterilisation of blood cultures. All of these criteria will be

assessed on day 4, counted from the day of randomisation (day 1) in order to determine a rapid response from the trial drug.

- (3) Microbiologic resolution of infection – defined as sterility of blood cultures collected on or before day 4
- (4) Microbiologic relapse – defined as growth of the same organism as in the original blood culture after the end of the period of study drug administration but before day 30
- (5) Superinfection with a carbapenem or piperacillin-tazobactam resistant organism or *Clostridium difficile* - defined as growth of a meropenem or piperacillin-tazobactam resistant organism from any clinical specimen collected from day 4 of study drug administration to day 30 or a positive *C. difficile* stool test. This endpoint is important since one of the purposes of establishing an alternative to carbapenem therapy is to reduce infections with resistant organisms or *C. difficile*.

Interim Analyses

Interim analyses were initially planned for this trial after enrolment of 50, 100, 200 and 300 patients. A separate data safety and monitoring board (DSMB) is responsible for the safety assessment of the trial during its conduct. Following the first interim analysis (at 50 patients), the DSMB recommended for less frequent review at 50, 150 and 340 (75%) patients recruited.

2 ANALYSIS DATA SETS

Analyses will be conducted on the following data sets:

2.1 Modified Intention to Treat (mITT) Analysis Data Set

The mITT data set will include all randomised study participants who received at least one dose of allocated study drug regardless of their compliance with the rules of the study. The mITT data set will be used for the analyses of all primary and secondary end points. All safety-related analyses will be based on the mITT population.

2.2 Per Protocol (PP) Analysis Data Set

Participants who were randomised but did not receive the full duration of study medication according to the protocol are excluded from the PP analyses. The PP data set includes participants who were still receiving study medication on day 4. The PP population will also exclude patients with significant variations from the study protocol (e.g. receiving a second gram-negative active agent in days 1 to 5 post randomisation, receiving an incorrect dose of study drug, ceasing the study drug early or withdrawal from the study for any other reason). Minor procedural variations (e.g. failing to collect day 3 blood culture while afebrile) will not preclude patients from the PP analysis.

2.3 Protocol Violations

All protocol violations occurring after randomisation will be listed in the Clinical Study Report (CSR), tabulated by Study Subject ID and study centre. The final assignment of participants to the PP analysis population will be made at a protocol violation review meeting prior to database lock, with assessors blinded to the patient's primary outcome.

3 STATISTICAL METHODOLOGY

The major sets of analyses to be performed are:

1. Primary efficacy analysis: Statistical analytic strategy relating to the primary hypothesis.
2. Analysis of secondary efficacy endpoints: Statistical analytic strategy relating to the secondary efficacy endpoints.

The statistical methodologies for each set of analyses are described below, unless noted otherwise.

3.1 Data Export and Archive

The study database is maintained at the Queensland Clinical Trials & Biostatistics Centre (QCTBC) of The University of Queensland. All data queries and corrections will be jointly conducted by the QCTBC and the MERINO study team prior to database lock. The QCTBC will manage the data and will conduct quality control of the data following their own standard operating procedures. All analyses performed, the Clinical Study Report(s) and the final data set will be archived together according to QCTBC standard operating procedures and the guidelines of The University of Queensland.

3.2 Data Validation

Data received by the QCTBC Statistician will be examined for missing values and outliers. Measures of central tendency and dispersion for continuous study parameters will be portrayed along with Box and Whisker plots. Extreme or unexpected values will be examined individually for authenticity and data discrepancies addressed where appropriate. Additional audit and statistical checks will be performed as necessary.

3.3 Imputation of Missing Data

For the measurement of daily monitoring variables contributing to the secondary outcome (e.g. resolution of leucocytosis), missing variables may be expected owing to variation in clinical care and omissions. For every day where a value is missing, the last measured value will be carried forward for each day until either a new value is recorded, the patient withdraws from the study or the patient dies, unless the outcome has already been reached (e.g. white cell count <12). Patients who fail to have a day 3 blood culture collected but were otherwise afebrile (temp <38°C) will be assumed to have achieved microbiological resolution (i.e. negative blood cultures). Otherwise, no imputation of missing data will be conducted. Missing data for relevant study parameters, if any, will be presented in accordance with standard procedures and compared across study arms.

3.4 Primary Efficacy Endpoint

3.4.1 Comparison of mortality by Day 30

The primary aim of this study is to compare the effects of two antibiotics on mortality on or before day 30. Since this is a non-inferiority study both the mITT population and the PP population will be used for this analysis. Non-inferiority must be met for analysis of the mITT population for piperacillin/tazobactam to be regarded as non-inferior to meropenem, with the findings in the PP population seen to be consistent in terms of direction and effect size estimates.

Mortality occurring at any time from randomisation up to and including day 30 will be rated as a binary variable (Yes/No). Risk ratios (RRs) and the absolute risk difference for mortality (with 95% CIs), with the meropenem arm as the reference group, will be calculated and represented by the use of forest plots in comparison to the non-inferiority margin, using the

Miettinen-Nurminen method. The proportion of patients [n (%)] who died will be reported for both treatment groups.

3.5 Secondary Efficacy Endpoints

Both the mITT and PP approaches will be used for the analyses of all secondary efficacy endpoints.

3.5.1 Time to clinical and microbiologic resolution of infection – the time to clinical and microbiologic resolution is the ‘first’ day on which fever is not present, the white cell count has normalised (total WCC $\leq 12 \times 10^9/L$) and blood cultures are not positive. The median number of days from randomisation to clinical and microbiologic resolution will be compared between the two treatment groups using the Wilcoxon Rank-sum test. The time to clinical and microbiologic resolution and 95% confidence interval of the median will be presented (also using boxplots) comparing each treatment arm. The day of clinical and microbiological resolution will be compared graphically by proportions of patients achieving this endpoint from days 1 to 5 for each treatment arm.

3.5.2 Clinical and Microbiologic Success – defined as survival at any time on day 4 PLUS resolution of fever on or before day 4 PLUS sterilisation of blood cultures collected on or before day 4

Clinical and microbiologic success at day 4 will be rated as a binary variable (Yes/No). Risk ratios (RRs) and the absolute risk difference for achieving this endpoint (with 95% CIs), with the meropenem arm as the reference group, will be calculated and represented by the use of forest plots. The proportion of patients [n (%)] who have clinical and microbiologic success will be reported for both treatment groups.

3.5.3 Microbiologic resolution of infection – defined as sterility of blood cultures collected on or before day 4

Microbiologic resolution will be rated as a binary variable (Yes/No). Risk ratios (RRs) and the absolute risk difference for achieving this endpoint (with 95% CIs), with the meropenem arm as the reference group, will be calculated and represented by the use of forest plots. The proportion of patients [n (%)] who have microbiologic resolution will be reported for both treatment groups.

3.5.4 Microbiologic relapse – defined as growth of the same organism as in the original blood culture after the end of the period of study drug administration but before 30 days after the first positive blood culture was drawn

Microbiologic relapse will be rated as a binary variable (Yes/No). Risk ratios (RRs) and the absolute risk difference for achieving this endpoint (with 95% CIs), with the meropenem arm as the reference group, will be calculated and represented by the use of forest plots. If the total number of relapses are insufficient, then 95% CIs will be omitted. The proportion of patients [n (%)] who have microbiologic relapse will be reported for both treatment groups.

3.5.5 Superinfection with a carbapenem resistant organism – defined as growth of a meropenem resistant Gram negative bacillus from any clinical specimen collected from the time of randomisation to day 30

Superinfection with a carbapenem resistant organism will be rated as a binary variable (Yes/No). Risk ratios (RRs) and the absolute risk difference for achieving this endpoint (with 95% CIs), with the meropenem arm as the reference group, will be calculated and represented by the use of forest plots. If the total number of superinfections are insufficient, then 95% CIs will be omitted. The proportion of patients [n (%)] who have superinfection will be reported for both treatment groups.

3.6 Subgroup analyses

An analysis of the primary and secondary efficacy endpoints as described above is proposed in the following subgroups, which is that of “high risk patients” defined by any of the following:

- (1) likely source of infection other than urinary tract,
- (2) Pitt score ≥ 4 on day 1

The Pitt bacteremia score will be calculated using the following criteria:

- oral temperature: 2 points for a temperature of $\leq 35^{\circ}\text{C}$ or $\geq 40^{\circ}\text{C}$, 1 point for a temperature of $35.1\text{--}36.0^{\circ}\text{C}$ or $39.0\text{--}39.9^{\circ}\text{C}$, and 0 points for a temperature of $36.1\text{--}38.9^{\circ}\text{C}$;

- hypotension: 2 points for an acute hypotensive event with decreases in systolic and diastolic blood pressure of 130 and 120 mm Hg, respectively, use of intravenous vasopressor agents, or systolic blood pressure <90 mm Hg;
- receipt of mechanical ventilation: 2 points;
- cardiac arrest: 4 points;
- mental status: alert, 0 points; disoriented, 1 point; stuporous, 2 points; and comatose, 4 points.

We will also compare primary outcomes for each treatment arm in patients where empirical antibiotics were congruent with subsequent randomisation allocation (e.g. received piperacillin-tazobactam or meropenem monotherapy and then were randomised to continue on the same drug), and when they were incongruent.

Additional sub-group analyses will be made for the primary outcome with respect to:

1. Region (Australia / New Zealand / Canada; Singapore; Europe; South Africa; Middle East)
2. Infecting genus (*E. coli* vs. *Klebsiella* spp)
3. Appropriate vs inappropriate empirical therapy*
4. Community vs. healthcare-associated infection

*Appropriate antibiotic therapy will be defined as: any agent started within 24h of initial blood culture collection to which the blood isolate was subsequently found to be susceptible *in vitro*, according to local laboratory standards. This will include combination therapy if at least one agent was appropriate.

7 BASIC STATISTICS IN THE CLINICAL STUDY REPORT

The basic statistics specified in the CSR will consist of the following:

- Information on missing values for all relevant study variables
- Summary of baseline categorical study variables with n (%); minimum, maximum, mean, standard deviation and first, second & third quartiles for continuous study variables
- Presentation by sub-categories including treatment allocation and study centre where appropriate

- For continuous study variables, box plots and Kernel density plots will also be provided as appropriate.

References

1. Harris PN, Peleg AY, Iredell J et al. Meropenem versus piperacillin-tazobactam for definitive treatment of bloodstream infections due to ceftriaxone non-susceptible *Escherichia coli* and *Klebsiella* spp (the MERINO trial): study protocol for a randomised controlled trial. *Trials* 2015; 16: 24.

Summary of Statistical Analysis Plan (SAP) amendments

Date	Summary of changes
10 th Feb 2013	SAP version 1
11 th Feb 2014	First Patient enrolled (Singapore)
3 rd Nov 2014	Amendments <ul style="list-style-type: none"> • Clarified definitions of secondary outcomes to align with published protocol • Definition of planned timings for interim analyses • Modification of planned sub-group analysis to include <ul style="list-style-type: none"> ○ Inappropriate vs appropriate empirical therapy ○ High-risk patients (source other than UTI, Pitt score ≥ 4) ○ Patients randomized to same class of antibiotic as empirical therapy
10 th Feb 2015	1 st DSMB interim analysis (50 patients randomized)
	<ul style="list-style-type: none"> • Timing of further interim analyses modified on recommendation of DSMB • On recommendation of DSMB, reporting of differences outcomes changed from odds-ratios to absolute risk differences and risk ratios
24 th June 2016	2nd DSMB interim analysis (150 patients randomized)
26 th April 2017 (Final version)	<ul style="list-style-type: none"> • Definition of modified ITT population • Addition of plan for handling missing data in secondary outcomes • Confidence intervals for risk differences calculated using the Miettinen-Nurminen method • Comparison of outcome 2.1 changed from using Mann-Whitney U test to Wilcoxon rank-sum • Addition of graphical representation for outcome 2.1 • Addition of sub-group analysis according to region of recruitment, Infecting genus, community versus healthcare associated infection • Addition of blinded assessment of PP population
26 th June 2017	3 rd DSMB interim analysis (340 patients randomized)